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Three novel species of *Helminthosporium* (Massarinaceae, Pleosporales) from China

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Abstract

Three new species of *Helminthosporium*, *H. nabanhensis*, *H. sinensis* and *H. yunnanensis* collected on dead branches of unidentified plants in Xishuangbanna, China, were proposed by morphological and molecular phylogenetic analysis. Phylogenetic analysis of the combined data of ITS-SSU-LSU-*TEF1-RPB2* sequences was performed using Maximum-Likelihood and Bayesian Inference, although *H. nabanhensis* and *H. sinensis* lack the *RPB2* sequences. Both molecular analyses and morphological data supported *H. nabanhensis*, *H. sinensis* and *H. yunnanensis* as three independent taxa within the Massarinaceae.

Keywords

asexual Ascomycota, hyphomycetes, lignicolous fungi, phylogenetic analysis, taxonomy

Introduction

Helminthosporium Link was originally erected by Link (1809) with *H. velutinum* as the type species, and was mainly characterized by macronematous, cylindrical, septate conidiophores with polytretic conidiogenous cells that producing solitary (rarely in short chains), acropleurogenous, clavate or obclavate, distoseptate conidia with a flat, ringed pore at the base (Ellis 1961, 1971; Luttrell 1964; Seifert

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et al. 2011). The genus became a repository for a large amount of species due to a lack of understanding of the generic concepts. To date, about 770 epithets for *Helminthosporium* are listed in Index Fungorum (2022), but most of these were not congeneric with the generic type in development of conidia and conidiophores. Ellis (1961) provided a review on *Helminthosporium*, and accepted ten species. Luttrell (1963, 1964) examined the type species and defined the generic concept, and Sivanesan (1987) transferred several unrelated pathogens of the *Poaceae* from *Helminthosporium* to the genera *Cochliobolus* (anamorph *Bipolaris*), *Setosphaeria* (anamorph *Exserohilum*) and *Pyrenophora* (anamorph *Drechslera*). Siboe et al. (1999) subsequently provided a synoptic table of the main morphological features that distinguish 27 accepted *Helminthosporium* species. Since then, 27 additional species have been described in the genus (Zhang et al. 2004, 2007, 2010; Shirouzu and Harada 2008; Zhang and Zhang 2009; Zhang and Sun 2010; Zhao and Zhao 2012; Wang et al. 2014; Tanaka et al. 2015; Zhu et al. 2016; Alves-Barbosa et al. 2017; Tian et al. 2017; Crous et al. 2018, 2019; Zhao et al. 2018; Boonmee et al. 2021; Chen et al. 2022). Voglmayr and Jaklitsch (2017) revealed the phylogenetic relationships of *Corynespora*, *Exosporium* and *Helminthosporium* species, synonymized *Exosporium* with *Helminthosporium*, and confirmed 17 species in *Helminthosporium* by morphological and molecular systematic analysis, but the generic concept has been widened by adding four *Corynespora* species that produce terminal, monotretic conidiogenous cells. So it is challenging to classify *Corynespora* and *Helminthosporium* species based on morphology alone because the distinction between monotretic vs. polytretic conidiogenous cells is the only character for separating *Corynespora* and *Helminthosporium*. Based on the records of Species Fungorum 2021, Konta et al. (2021) summarized the morphology, host information, locality, sequence data and related references of 216 *Helminthosporium* species reported worldwide. Unfortunately, sequence data for most species are unavailable, and only 27 species are represented by the DNA sequence in GenBank (Chen et al. 2022).

Helminthosporium is worldwide in distribution, usually found as a common saprobe on leaf or twig litter, but one specie, *H. solani*, is an economically important pathogen causing silver scurf disease in potatoes worldwide (Alcorn 1983; Voglmayr and Jaklitsch 2017; Boonmee et al. 2021). To date, only 28 species have been recorded in China, viz. *H. aquaticum*, *H. bambusicola*, *H. cantonense*, *H. chengduense*, *H. chinense*, *H. citri*, *H. conidiophorellum*, *H. constrictum*, *H. corchori*, *H. dongxingense*, *H. guangxiense*, *H. hunanense*, *H. ipomoeae*, *H. juglandis*, *H. lablab*, *H. ligustri*, *H. marantae*, *H. multiseptatum*, *H. nanjingense*, *H. obpyriforme*, *H. oplismeni*, *H. ovoideum*, *H. piperis*, *H. pseudomicrosorum*, *H. rhodomyrti*, *H. sichuanense*, *H. subhyalinum* and *H. submersum* (Zhang et al. 2004; Zhang and Zhang 2009; Zhang et al. 2010; Zhang and Sun 2010; Zhao and Zhao 2012; Wang et al. 2014; Zhu et al. 2016; Zhao et al. 2018; Chen et al. 2022).

Xishuangbanna lies on the northern edge of tropical Southeast Asia. It is located in the southwestern part of Yunnan Province, China. It covers 19,125 km^2 and has a mountainous topography and humid tropical monsoon climate, with an av-

erage annual temperature of 19.3–23.9 °C, and an average annual precipitation of 1200–1800 mm. The primary forest vegetation types are tropical seasonal rain forest, tropical montane rain forest, evergreen broad-leaved forest, monsoon forest over limestone, and monsoon forest on river banks (Cao and Zhang 1997). Such conditions create a very wide range of habitats favoring the growth of various microbial species. During our continuing mycological surveys of saprobic microfungi from plant debris in this region, three interesting hyphomycetes with morphological features typical of *Helminthosporium* were collected on dead branches. Based on morphological data and multi-locus phylogenetic analysis, they were described as new to science in the present study.

Materials and methods

Sample collection, isolation and morphological studies

Samples of dead branches were collected from humid environments and river banks in the forest ecosystems of Xishuangbanna, Yunnan Province, China, and returned to the laboratory in Ziploc bags. Samples were processed and examined following the methods described in Ma et al. (2011). Fungi were mounted in a drop of lactic acid on microscope slides, and examined and photographed with an Olympus microscope (model BX 53), with a 100 × (oil immersion) objective at the same background color and scale. Adobe Photoshop 7.0 was used for image processing to assemble photographs into images. Single-spore isolations were made on potato dextrose agar (PDA) following Goh (1999). Colony colors were assessed according to the charts of Rayner (1970). All fungal strains were stored in 10% sterilized glycerin at 4 °C for further studies. The studied specimens and cultures were deposited in the Herbarium of Jiangxi Agricultural University, Plant Pathology, Nanchang, China (**HJAUP**).

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from fungal mycelia grown on PDA, using the Solarbio Fungi Genomic DNA Extraction Kit following the manufacturer's protocol (Solarbio, China). The DNA amplification was performed by polymerase chain reaction (PCR) using the respective loci (ITS, SSU, LSU, *TEF1*, *RPB2*). Primer sets used for these genes were as follows: ITS: ITS5/ITS4 (White et al. 1990), SSU: 18S-F/18S-R, LSU: 28S1-F/28S3-R (Xia et al. 2017), *TEF1*: EF1-983F/EF1-2218R (Rehner 2001; Zhao et al. 2018) and *RPB2*: dRPB2-5f/dRPB2-7r (Voglmayr et al. 2016). The final volume of the PCR reaction was 25 μl, containing 1 μl of DNA template, 1 μl each of the forward and reverse primer, 12.5 μl of 2 × Power Taq PCR MasterMix and 9.5 μl of double-distilled water (ddH_2O). The PCR thermal cycling conditions of ITS, SSU and LSU were initialized at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 50 s, elongation at 72 °C

for 1 min, a final extension at 72 °C for 10 min, and finally kept at 4 °C, the *TEF1* and *RPB2* were initialized at 95 °C for 3 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at a suitable temperature for 30 s, elongation at 72 °C for 1 min, a final extension at 72 °C for 10 min, and finally kept at 4 °C. Annealing temperature was 60 °C for *TEF1*, 56 °C for *RPB2*. The PCR products were checked on 1% agarose gel electrophoresis stained with ethidium bromide. Purification and DNA sequencing were carried out at Beijing Tsingke Biotechnology Co., Ltd. China.

Sequence alignment and phylogenetic analysis

The newly generated sequences together with other sequences obtained from GenBank (Table 1) were initially aligned using MAFFTv.7 (Katoh and Standley 2013) on the online server (http://maffTh.cbrc.jp/alignment/server/), and optimized manually when needed. To establish the identity of the isolates at species level, phylogenetic analyses were conducted first individually for each locus and then as combined analyses of five gene loci (ITS, LSU, SSU, *TEF1* and *RPB2*). Five aligned data sets of ITS, LSU, SSU, *TEF1* and *RPB2* are concatenated using the concatenated sequence function of Phylosuite software v1.2.1 (Zhang et al. 2020a), and absent sequence data (i.e., ITS, LSU, SSU, *TEF1* and *RPB2* sequence data) in the alignments were treated with the question mark as missing data. Phylosuite software v1.2.1 (Zhang et al. 2020a) was used to construct the phylogenetic tree based on ITS, SSU, LSU, *TEF1* and *RPB2* sequence data. The concatenated aligned dataset was analyzed separately using Maximum likelihood (ML) and Bayesian inference (BI). Maximum likelihood phylogenies were inferred using IQ-TREE (Nguyen et al. 2015) under Edge-linked partition model for 10000 ultrafast bootstraps (Hoang et al. 2017). The final tree was selected among suboptimal trees from each run by comparing the likelihood scores using the TIM2e+I+G4 for ITS+*RBP2*, TVMe+I+G4 for LSU+SSU, and TNe+R2 for *TEF1* substitution model. Bayesian Inference phylogenies were inferred using MrBayes 3.2.6 (Ronquist et al. 2012) under partition model (2 parallel runs, 2000000 generations), in which the initial 25% of sampled data were discarded as burn-in. The best-fit model was SYM+I+G4 for ITS+*RBP2*, LSU+SSU; SYM+G4 for *TEF1*. ModelFinder (Kalyaanamoorthy et al. 2017) was used to select the best-fit partition model (Edge-linked) using BIC criterion. The trees were viewed in FigTree v. 1.4.4 (http://tree.bio.ed.ac.uk/software/figtree) and further edited in Adobe Illustrator 2021.

Results

Molecular phylogeny

Three new strains of *Helminthosporium* isolated from dead branches in Xishuangbanna, Yunnan Province, China, were grown in culture and used for analyses of molecular

Table 1. Species and GenBank accession numbers of DNA sequences used in this study. New sequences are in bold.

Taxon	Strain	Genbank accession numbers				
		SSU	LSU	ITS	*RPB2*	*TEF1*
Byssothecium circinans	CBS 675.92	GU205235	GU205217	OM337536	DQ767646	GU349061
Corynespora cassiicola	CBS 100822	GU296144	GU301808	–	GU371742	GU349052
Corynespora smithii	L120	–	KY984297	KY984297	KY984361	KY984435
Corynespora smithii	L130	KY984419	KY984298	KY984298	KY984362	KY984436
Cyclothyriella rubronotata	TR, CBS 121892	–	KX650541	KX650541	KX650571	KX650516
Cyclothyriella rubronotata	TR9, CBS 141486	KX650507	KX650544	KX650544	KX650574	KX650519
Helminthosporium aquaticum	MFLUCC 15-0357, S-096[HT]	KU697310	KU697306	KU697302	–	–
Helminthosporium austriacum	L132[HT], CBS 139924	KY984420	KY984301	KY984301	KY984365	KY984437
Helminthosporium austriacum	L137	–	KY984302	KY984302	KY984366	KY984438
Helminthosporium austriacum	L169, CBS 142388	–	KY984303	KY984303	KY984367	KY984439
Helminthosporium caespitosum	L141	–	KY984305	KY984305	KY984368	–
Helminthosporium caespitosum	L151	–	KY984306	KY984306	KY984369	–
Helminthosporium caespitosum	L99[HT], CBS 484.77	KY984421	JQ044448	JQ044429	KY984370	KY984440
Helminthosporium chengduense	UESTC 22.0024, CGMCC 3.23575[HT]	ON557757	ON557745	ON557751	ON563073	ON600598
Helminthosporium chengduense	UESTC 22.0025	ON557756	ON557744	ON557750	ON563072	ON600597
Helminthosporium chiangraiense	MFLUCC 21-0087[HT]	–	MZ538538	MZ538504	–	–
Helminthosporium chinense	UESTCC 22.0026, CGMCC 3.23570[HT]	ON557760	ON557748	ON557754	–	ON600601
Helminthosporium chlorophorae	BRIP 14521	–	–	AF120259	–	–
Helminthosporium dalbergiae	H 4628, MAFF 243853	AB797231	AB807521	LC014555	–	AB808497
Helminthosporium endiandrae	CBS 138902, CPC 22194[HT]	–	KP004478	KP004450	–	–
Helminthosporium erythrinicola	CBS 145569[HT]	–	MK876432	NR_165563	MK876486	–
Helminthosporium genistae	L128, CBS 139921	KY984422	KY984308	KY984308	KY984372	–
Helminthosporium genistae	L129, CBS 139922	KY984423	KY984309	KY984309	KY984373	–
Helminthosporium genistae	L142[ET], CBS 142597	–	KY984310	KY984310	KY984374	–
Helminthosporium hispanicum	L109[HT], CBS 136917	KY984424	KY984318	KY984318	KY984381	KY984441
Helminthosporium italicum	MFLUCC 17-0241	–	KY815015	KY797638	–	KY815021
Helminthosporium juglandinum	L118[HT], CBS 136922	–	KY984321	KY984321	KY984384	KY984444
Helminthosporium juglandinum	L97, CBS 136911	KY984425	KY984322	KY984322	KY984385	KY984445
Helminthosporium leucadendri	CBS 135133, CPC 19345[HT]	–	KF251654	KF251150	KF252159	KF253110
Helminthosporium livistonae	CPC 32158, CBS 144413[HT]	–	NG_064539	NR_160348	–	–
Helminthosporium magnisporum	H 4627, MAFF 239278, TS 33[HT]	AB797232	AB807522	AB811452	–	AB808498
Helminthosporium massarinum	KT 1564[HT], CBS 139690	AB797234	AB807524	AB809629	–	AB808500
Helminthosporium massarinum	KT 838[EP], MAFF 239604	AB797233	AB807523	AB809628	–	AB808499
Helminthosporium microsorum	L94	KY984426	KY984327	KY984327	KY984388	KY984446
Helminthosporium microsorum	L95	–	KY984328	KY984328	KY984389	KY984447
Helminthosporium microsorum	L96[ET], CBS 136910	KY984427	KY984329	KY984329	KY984390	KY984448
Helminthosporium nabanhensis	**HJAUP C2054[ET]**	**OP555400**	**OP555398**	**OP555394**	–	**OP961931**
Helminthosporium nanjingensis	ZM020380	–	–	KF192322	–	–
Helminthosporium oligosporum	L106	–	KY984330	KY984330	KY984391	KY984449
Helminthosporium oligosporum	L92, CBS 136908	KY984428	KY984332	KY984332	KY984393	KY984450
Helminthosporium oligosporum	L93[ET], CBS 136909	–	KY984333	KY984333	KY984394	KY984451
Helminthosporium quercinum	L90[HT], CBS 136921	KY984429	KY984339	KY984339	KY984400	KY984453
Helminthosporium quercinum	L91	–	KY984340	KY984340	KY984401	KY984454
Helminthosporium sinensis	**HJAUP C2121[ET]**	**OP555399**	**OP555397**	**OP555393**	–	**OP961932**
Helminthosporium solani	CBS 365.75	KY984430	KY984341	KY984341	KY984402	KY984455
Helminthosporium solani	CBS 640.85	–	KY984342	KY984342	KY984403	–
Helminthosporium submersum	UESTCC 22.0021	ON557759	ON557747	ON557753	ON563075	ON600600
Helminthosporium submersum	MFLUCC 16-1360[HT]	MG098796	MG098787	–	–	MG098586
Helminthosporium submersum	MFLUCC 16-1290[PT]	MG098797	MG098788	MG098780	MG098592	MG098587

Taxon	Strain	Genbank accession numbers				
		SSU	LSU	ITS	*RPB2*	*TEF1*
Helminthosporium syzygii	CBS 145570 HT	–	MK876433	NR_165564	MK876487	–
Helminthosporium tiliae	L171	–	KY984343	KY984343	KY984404	KY984456
Helminthosporium tiliae	L88 ET, CBS 136907	KY984431	KY984345	KY984345	KY984406	KY984457
Helminthosporium tiliae	L89	–	KY984346	KY984346	KY984407	–
Helminthosporium velutinum	H 4626, MAFF 243854	AB797240	AB807530	LC014556	–	AB808505
Helminthosporium velutinum	H 4739, MAFF 243855	AB797235	AB807525	LC014557	–	AB808501
Helminthosporium velutinum	L115, CBS 136924	–	KY984347	KY984347	KY984408	KY984458
Helminthosporium velutinum	L116	–	KY984348	KY984348	KY984409	KY984459
Helminthosporium velutinum	L117	–	KY984349	KY984349	KY984410	KY984460
Helminthosporium velutinum	L126	–	KY984350	KY984350	KY984411	KY984461
*Helminthosporium velutin*um	L127	–	KY984351	KY984351	KY984412	KY984462
Helminthosporium velutinum	L131 ET, CBS 139923	KY984432	KY984352	KY984352	KY984413	KY984463
Helminthosporium velutinum	L98	KY984433	KY984359	KY984359	KY984417	KY984466
Helminthosporium velutinum	yone 96, MAFF 243859	AB797239	AB807529	LC014558	–	AB808504
Helminthosporium yunnanensis	**HJAUP C2071 ET**	**OP555392**	**OP555396**	**OP555395**	**OP961934**	**OP961933**
Massarina cisti	CBS 266.62, JCM 14140 HT	AB797249	AB807539	LC014568	FJ795464	AB808514
Massarina eburnea	CBS 473.64	AF164367	GU301840	AF383959	GU371732	GU349040
Massarina eburnea	H 3953, CBS 139697	AB521718	AB521735	LC014569	–	AB808517
Periconia byssoides	H 4600, MAFF 243872	AB797280	AB807570	LC014581	–	AB808546
Periconia digitata	CBS 510.77	AB797271	AB807561	LC014584	–	AB808537
Periconia pseudodigitata	KT 1395, CBS 139699, MAFF 239676 HT	NG_064850	NG_059396	NR_153490	–	AB808540
Pseudosplanchnonema phorcioides	L16, CBS 122935	KY984434	KY984360	KY984360	KY984418	KY984467
Stagonospora paludosa	CBS 135088, S601 NT	–	KF251760	KF251257	KF252262	KF253207
Stagonospora perfecta	KT 1726A, MAFF 239609	AB797289	AB807579	AB809642	–	AB808555
Stagonospora pseudoperfecta	KT 889, CBS 120236, MAFF 239607 HT	AB797287	AB807577	AB809641	–	AB808553
Stagonospora tainanensis	KT 1866, MAFF 243860	AB797290	AB807580	AB809643	–	AB808556

[1]"–", sequence is unavailable.

[2]Strain with ET (epitype), HT (holotype), NT (neotype), and PT (paratype).

[3]Abbreviations: **CBS:** Central Bureau voor Schimmel cultures, Utrecht, The Netherlands; **CGMCC:** China General Microbiological Culture Collection Center; **CPC:** Collection of Pedro Crous housed at CBS; **HJAUP:** Herbarium of Jiangxi Agricultural University, Plant Pathology; **MAFF:** the National Institute of Agrobiological Sciences, Japan; **MFLUCC:** Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; **UESTCC:** The University of Electronic Science and Technology Culture Collection, Chengdu, China; **ITS:** Internal Transcribed Spacer; **SSU:** Small Subunit Ribosomal; **LSU:** Large Subunit Ribosomal; ***TEF1*:** Transcriptional Enhancer Factor 1-alpha; ***RPB2*:** The Second Largest Subunit of RNA Polymerase II; others are not registered abbreviations.

sequence data. Unfortunately, our two species, *H. nabanhensis* and *H. sinensis* lack the *RPB2* sequences. Newly generated sequences were deposited in GenBank. Alignment has 75 sequences with 1511 total characters (The combined dataset, ITS:1–457, LSU:458–993, *RBP2*:994–1110, SSU:1111–1363, *TEF1*:1364–1511), 555 distinct patterns, 487 parsimony-informative, 89 singleton sites, 935 constant sites, and *Cyclothyriella rubronotata* (TR) and *C. rubronotata* (TR9) were regarded as an outgroup. Maximum likelihood and Bayesian Inference analyses of the combined dataset resulted in phylogenetic reconstructions with largely similar topologies, and bootstrap support values for Maximum likelihood higher than 90% and Bayesian posterior probabilities greater than 0.90 are given above the nodes. The best-scoring ML consensus tree (lnL = –10,686.191) with ultrafast bootstrap values from ML analyses and posterior probabilities from MrBayes analysis at the nodes are shown in Fig. 1. *Helminthosporium nabanhensis* form a distinct clade sister to *H. chlorophorae* with

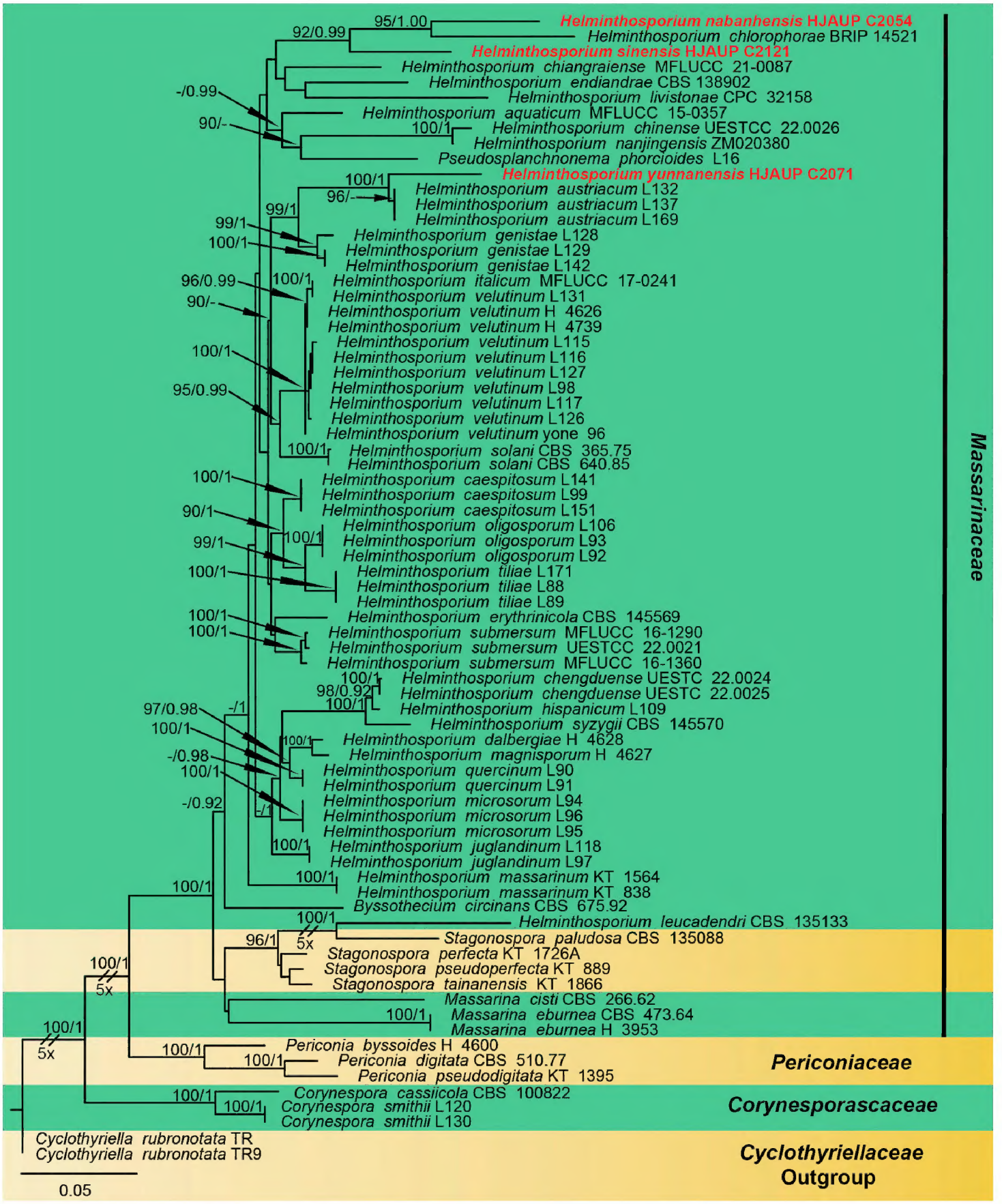

Figure 1. Phylogram of Massarinaceae based on combined ITS, SSU, LSU, *RPB2* and *TEF1* sequences. The ML and BI bootstrap support values above 90% and 0.90 are shown at the first and second position, respectively. The tree is rooted to *Cyclothyriella rubronotata* (TR) and *C. rubronotata* (TR9). Strains from the current study are in red. Some branches were shortened according to the indicated multipliers.

strong statistical support (ML/BI = 95/1.00); *H. sinensis* forms a high-support clade (ML/BI = 92/0.99) with the lineage consisting of *H. nabanhensis* and *H. chlorophorae*; *H. yunnanensis* is a sister to three different strains of *H. austriacum* with strong statistical support (ML/BI = 100/1.00).

Taxonomy

Helminthosporium nabanhensis **Jing W. Liu & Jian Ma, sp. nov.**
IndexFungorum No: 559980
Fig. 2

Etymology. Referring to the collecting site of Nabanhe Nature Reserve in Yunnan Province, China.

Holotypus. HJAUP M2054.

Description. Saprobic on dead branches. ***Sexual morph*:** Undetermined. ***Asexual morph*:** Hyphomycetous. ***Colonies*** on natural substrate effuse, scattered, hairy, brown to black. ***Mycelium*** partly superficial, partly immersed in the substratum, composed of branched, septate, pale brown to brown, smooth hyphae. ***Conidiophores*** macronematous, mononematous, solitary or in groups of 2–4, simple, occasionally branched, erect, straight or flexuous, cylindrical, smooth, 8–21-septate, brown to dark brown, paler towards the apex, with well-defined small pores at the apex and rarely laterally beneath the upper 1–3 septa, 365–557 × 6.5–13.5 μm. ***Conidiogenous cells*** polytretic, integrated, terminal and intercalary, cylindrical, brown, smooth, with noncicatrized, distinct pores. Conidial secession schizolytic. ***Conidia*** acropleurogenous, solitary, dry, obclavate, pale brown to brown, 3–6-distoseptate, smooth, straight or curved, wider below than apex, truncate and dark at base, apically rostrate and pale, guttulate when young, non-guttulate at maturity, 26.5–46.5 μm long, 6.5–10 μm wide, tapering to 3–3.5 μm wide near the apex, 3–6 μm wide at the basal scar.

Cultural characteristics. Colony on PDA reaching 50–55 mm diam. after 2 weeks in an incubator under dark conditions at 25 °C, irregular circular, surface velvety, with white and denser mycelium at the center, becoming olivaceous and sparser towards the edge; reverse pale brown at the center, dark brown at the periphery.

Material examined. China, Yunnan Province: Xishuangbanna Dai Autonomous Prefecture, Nabanhe National Nature Reserve, on dead branches of an unidentified broadleaf tree, 12 July 2021, J.W. Liu, HJAUP M2054 (Holotype), ex-type living culture HJAUP C2054.

Notes. The phylogenetic tree shows that the strain of *H. nabanhensis* (HJAUP C2054) clusters with the ex-type strain of *H. chlorophorae* (BRIP 14521). The BLASTn analysis of ITS of our ex-type strain HJAUP C2054 showed 90% identity (425/471 bp, 10/471 gaps) with ex-type strain BRIP 14521 of *H. chlorophorae*. Moreover, *H. nabanhensis* morphologically differs from *H. chlorophorae* in bigger conidiophores (365–557 × 6.5–13.5 μm vs. 120–270 × 7–10 μm) occasionally branched, and smaller conidia (26.5–46.5 × 6.5–10 μm vs. 52–102 × 8–11 μm) with fewer septa (3–6 vs. 6–9), and from *H. sichuanense* (Zhang et al. 2004) in narrower conidiophores (6.5–13.5 μm vs. 14–25 μm) and smaller conidia (26.5–46.5 × 6.5–10 μm vs. 41–86 × 10–14 μm) with fewer septa (3–6 vs. 5–11).

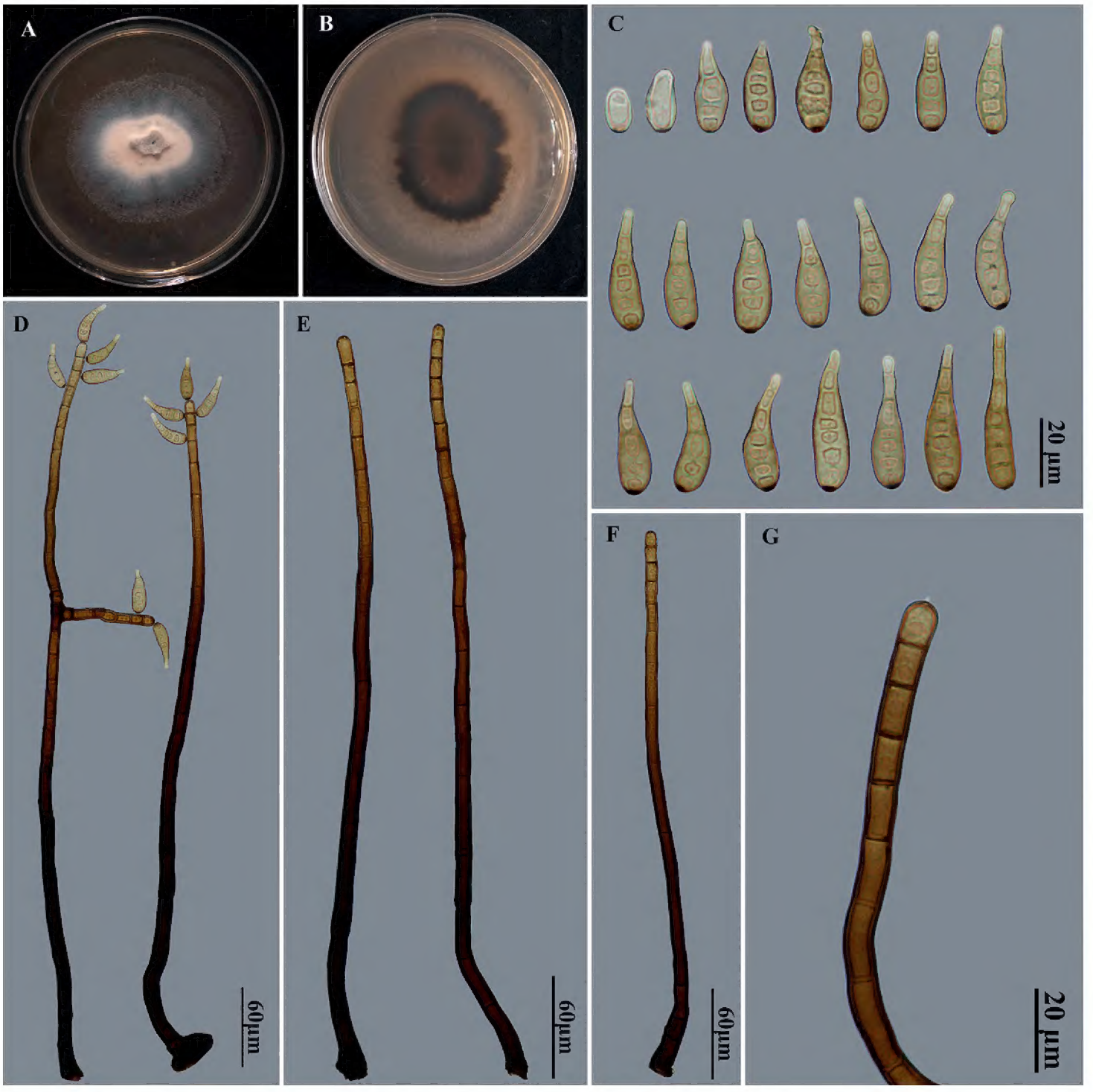

Figure 2. *Helminthosporium nabanhensis* (HJAUPM2054, holotype) **A, B** culture on PDA from above and reverse **C** conidia **D** conidiophores with conidia **E–G** conidiophores with conidiogenous cells.

Helminthosporium sinensis **Jing W. Liu & Jian Ma, sp. nov.**
IndexFungorum No: 559981
Fig. 3

Etymology. Referring to the country in which the fungus was collected.

Holotypus. HJAUP M2121.

Description. Saprobic on dead branches. ***Sexual morph*:** Undetermined. ***Asexual morph*:** Hyphomycetous. ***Colonies*** on natural substrate effuse, scattered, hairy, brown to black. ***Mycelium*** partly superficial, partly immersed in the substratum, composed of branched, septate, pale brown to brown, smooth hyphae.

Figure 3. *Helminthosporium sinensis* (HJAUPM2121, holotype) **A, B** culture on PDA from above and reverse **C** conidia **D, E** conidiophores, conidiogenous cells and conidia **F** conidiophores.

Conidiophores macronematous, mononematous, solitary or in groups of 2–4, simple, straight or flexuous, thick-walled, cylindrical, smooth, brown to dark brown, paler towards the apex, with well-defined small pores at the apex and rarely laterally beneath the upper 1–4 septa, 220–370 × 6–8.5 μm. ***Conidiogenous cells*** polytretic, integrated, terminal and intercalary, cylindrical, brown, smooth, with noncicatrized, distinct pores. Conidial secession schizolytic. ***Conidia*** acropleurogenous, solitary, rarely catenate, dry, obclavate, pale brown, 2–7-distoseptate, smooth, straight or curved, wider below than apex, truncate and dark at base, apically rostrate and pale, 37–60 μm long, 5.5–8.5 μm wide, tapering to 3–3.5 μm wide near the apex, 3–6 μm wide at the basal scar.

Cultural characteristics. Colony on PDA reaching 30–37 mm diam. after 2 weeks in an incubator under dark conditions at 25 °C, pale brown, irregular

circular, surface velvety, outermost layer gray; reverse dark brown, produces pale green pigment.

Material examined. China, Yunnan Province: Xishuangbanna Dai Autonomous Prefecture, Menghai County, Mengsong Township, on dead branches of an unidentified broadleaf tree, 13 July 2021, J.W. Liu, HJAUP M2121 (Holotype), ex-type living culture HJAUP C2121.

Notes. Phylogenetic analysis shows that the strain of *H. sinensis* (HJAUP C2121) forms an independent clade, and clusters with the strains of *H. nabanhensis* (HJAUP C2054) and *H. chlorophorae* (BRIP 14521). The BLASTn analysis of ITS of our ex-type strain HJAUP C2121 showed 89% identity (536/602 bp, 17/602 gaps) with ex-type strain HJAUP C2054 of *H. nabanhensis*, and showed 91% identity (430/471 bp, 13/471 gaps) with ex-type strain BRIP 14521 of *H. chlorophorae*. Moreover, *H. sinensis* differs from *H. nabanhensis* by its longer and narrower conidia (37–60 × 5.5–8.5 μm vs. 26.5–46.5 × 6.5–10 μm), and smaller conidiophores (220–370 × 6–8.5 μm vs. 365–557 × 6.5–13.5 μm), and from *H. chlorophorae* by its smaller conidia (37–60 × 5.5–8.5 μm vs. 52–102 × 8–11 μm) and longer and narrower conidiophores (220–370 × 6–8.5 μm vs. 120–270 × 7–10 μm), and from *H. guangxiense* (Zhang and Zhang 2009) in smaller conidiophores (220–370 × 6–8.5 μm vs. 330–850 × 14–25 μm) and smaller conidia (37–60 × 5.5–8.5 μm vs. 76–110 × 16–22 μm) with fewer septa (2–7 vs. 9–17). In addition, the conidia of *H. sinensis* are solitary or rarely catenate, whereas those of *H. guangxiense*, *H. nabanhensis* and *H. chlorophorae* are solitary.

Helminthosporium yunnanensis **Jing W. Liu & Jian Ma, sp. nov.**
IndexFungorum No: 559982
Fig. 4

Etymology. Referring to Yunnan province, where the type specimen was collected.

Holotypus. HJAUP M2071.

Description. Saprobic on dead branches. ***Sexual morph*:** Undetermined. ***Asexual morph*:** Hyphomycetous. ***Colonies*** on natural substrate effuse, scattered, hairy, brown to dark brown. Mycelium partly superficial, partly immersed in the substratum, composed of branched, septate, pale brown to brown, smooth hyphae. ***Conidiophores*** macronematous, mononematous, solitary or in groups of 2–4, simple, straight or flexuous, thick-walled, cylindrical, smooth, brown to dark brown, paler towards the apex, with one cylindrical, enteroblastic percurrent extension, and with well-defined small pores at the apex and rarely laterally beneath the upper 1–5 septa, 560–680 × 12.5–15.5 μm. ***Conidiogenous cells*** polytretic, integrated, terminal and intercalary, cylindrical, pale brown to brown, smooth, with noncicatrized, distinct pores. Conidial secession schizolytic. ***Conidia*** acropleurogenous, solitary, dry, obclavate, sigmoid, lunate or uncinate, pale brown, 4–7-distoseptate, smooth, straight or flexuous, wider below than apex, truncate and dark at base, apically rostrate and pale, 30.5–55.5 μm long, 9–11 μm wide, tapering to 2.5–3 μm near the apex, 3–7.5 μm wide at the basal scar.

Figure 4. *Helminthosporium yunnanensis* (HJAUPM2071, holotype) **A, B** culture on PDA from above and reverse **C** conidiophores with conidia **D** conidiogenous cells and conidia **E** conidia.

Cultural characteristics. Colony on PDA reaching 75–82 mm diam. after 2 weeks in an incubator under dark conditions at 25 °C, irregular circular, surface velvety, with brown and denser mycelium at the center, becoming white and sparser towards the edge; reverse pale brown at the center, with little black dots.

Material examined. China, Yunnan Province: Xishuangbanna Dai Autonomous Prefecture, Nabanhe National Nature Reserve, on dead branches of an unidentified broadleaf tree, 12 July 2021, J.W. Liu, HJAUP M2071 (Holotype), ex-type living culture HJAUP C2071.

Notes. Phylogenetic analysis shows that the strain of *H. yunnanensis* (HJAUP C2071) clustered together and formed a sister clade with three different strains of *H. austriacum* (L132, L137, L169) (Voglmayr and Jaklitsch 2017). The BLASTn analysis of *H. yunnanensis* (HJAUP C2071) and *H. austriacum* (L132^{HT}) shows 97% identity

(524/541, 4 gaps) using ITS, 99% identity (550/553, 2 gaps) using LSU, 99% identity (872/873, 1 gap) using SSU, 98% identity (738/752, no gap) using *TEF1*, and 98% identity (1077/1095, no gap) using *RPB2*. *Helminthosporium yunnanensis* morphologically differs from *H. austriacum* in wider conidiophores (560–680 × 12.5–15.5 μm vs. 275–700 × 7–11 μm) with one cylindrical, enteroblastic percurrent extension, and narrower conidia (30.5–55.5 × 9–11 μm vs. 35–48 × 13.7–16.5 μm), and from *H. obpyriforme* (Zhang and Zhang 2009) in bigger conidiophores (560–680 × 12.5–15.5 μm vs. 225–460 × 9.5–13 μm) and smaller conidia (30.5–55.5 × 9–11 μm vs. 47–74 × 14–19 μm) with fewer septa (4–7 vs. 5–9).

Discussion

The taxonomic history of the genus *Helminthosporium* is complex. To date, about 770 epithets for *Helminthosporium* are listed in Index Fungorum (2022), but most of these were not congeneric with the generic type. Konta et al. (2021) listed 216 *Helminthosporium* species based on records from Species Fungorum, but most species are identified based on morphological studies, and so far only 27 species are represented by a DNA sequence in GenBank (Voglmayr and Jaklitsch 2017; Boonmee et al. 2021; Chen et al. 2022). Morphological comparison is important for fungal identification, but species identification only based on morphological studies is not comprehensive. With the availability of supplementary sequence data for *Helminthosporium* species, the molecular phylogenetic analysis is being used to evaluate previously described *Helminthosporium*-like species by molecular methods. The introduction of a phylogenetic analysis of *Helminthosporium* led to a better improvement of the heterogeneity of the genus and further clarified the taxonomic status of *Helminthosporium*. Voglmayr and Jaklitsch (2017) revisited *Corynespora*, *Exosporium* and *Helminthosporium*, with phylogenetic and morphological analyses. Zhang et al. (2020b) transferred *H. bigenum* into a new genus *Mirohelminthosporium* K. Zhang, D.W. Li & R.F. Castañeda and replaced the illegitimate *H. cylindrosporum* Matsush. with *H. matsushimae*. Chen et al. (2022) suggested four *Helminthosporium* species, *H. anomalum*, *H. asterinum*, *H. decacuminatum* and *H. gibberosporum* to *Bipolaris*, *Kirschsteiniothelia* or *Curvularia* by performing blastn analysis. Furthermore, seven new species were described under the genus *Helminthosporium* by molecular methods (Crous et al. 2018, 2019; Zhao et al. 2018; Boonmee et al. 2021; Chen et al. 2022). Based on previous studies, we proposed three new species by morphological and molecular phylogenetic analysis.

Chen et al. (2022) described two new species, *H. chengduense* and *H. chinense*, based on combined ITS, LSU, SSU, *TEF1* and *RPB2* sequence data and morphological characters. Accordingly, we also used ITS, LSU, SSU, *TEF1* and *RPB2* for phylogenetic analysis and obtained high phylogenetic support, although our two species, *H. nabanhensis* and *H. sinensis*, lack the *RPB2* sequences. They are considerably distinct from all other described *Helminthosporium* species by morphological characters and multi-locus phylogenetic analysis, so we are convinced that the newly introduced species are new to science.

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